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Molecular Genetic and Biochemical Analyses of a DNA Repair Gene From Serratia Marcescens.

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Molecular genetic and biochemical analyses of a DNA repair gene from *Serratia marcescens*

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Ann Arbor, MI 48106

MOLECULAR GENETIC AND BIOCHEMICAL ANALYSES OF A DNA REPAIR GENE FROM *SERRATIA MARCESCENS*

A dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

in

The Department of Microbiology

(Interdepartmental Studies in Genetics)

by
Keith E. Murphy
B.S., Indiana University, 1982
M.S., University of Cincinnati
College of Medicine, 1985
August, 1989

DEDICATION

This work is dedicated to the memory of my father Stephen J. Murphy. While this world would not grant him the opportunity to realize his dreams, he and my mother unselfishly ensured that I would have such opportunities.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and gratitude to the following individuals.

Dr. H. Douglas Braymer, for his willingness to allow me to explore research interests distant from his own. The freedom given me most certainly stimulated my scientific creativity and productivity.

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K.E.M.

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ABSTRACT

In Escherichia coli, the SOS response and two 3-methyladenine DNA glycosylases (TagI and TagII) are required for repair of DNA damaged by alkylating agents such as methyl methanesulfonate (MMS). Mutations of the recA gene eliminate the SOS response. TagI and TagII are encoded by the tag and alkA genes, respectively. A gene (rpr) encoding 3-methyladenine DNA glycosylase activity was isolated from the Gram-negative bacterium Serratia marcescens. The gene, localized to a 1.5-kilobase pair SmaI-HindIII restriction fragment, was cloned into plasmid pUC18. The clone complemented E. coli tag alkA and recA mutations for MMS resistance. The rpr gene did not, however, complement recA mutations for resistance to ultraviolet light or the ability to perform homologous recombination reactions, nor did it complement E. coli ada or alkB mutations.

Two proteins of molecular weights 42,000 and 16,000 were produced from the rpr locus. Analysis of deletion and insertion mutants of rpr suggested that the 42kD molecule is the active protein. The 16kD protein may either be a breakdown product of the 42kD species or may be encoded by another gene overlapping the reading frame of the rpr gene. Biochemical assays showed that the rpr gene product (Rpr) possesses 3-methyladenine DNA glycosylase activity. Moreover, Rpr acts exclusively on 3-methyladenine, thus

exhibiting the same substrate specificity as *E. coli* TagI. Furthermore, Rpr was as effective as TagI in host cell reactivation of bacteriophage lambda which had been exposed to MMS.

Finally, *rpr* did not complement a triple mutation in *E. coli* (*tag alkA recA*) which abolished 3-methyladenine DNA glycosylase activity and the SOS response. Southern blot hybridization experiments evidenced no detectable homology between *rpr* and various known *E. coli* DNA repair genes. Furthermore, the *E. coli tag* and *alkA* genes did not hybridize to *S. marcescens* chromosomal DNA. Gene dosage experiments demonstrated no differences in *recA* complementation profiles whether *rpr* was on a low or high copy plasmid vector. However, *rpr* on the high copy vector pUC18 sensitized wild type *E. coli* and *E. coli* mutants deficient in exonuclease III (*E. coli xth*) and/or endonuclease IV (*E. coli xth nfo*) to MMS.

INTRODUCTION

Alkylation of DNA causes lesions that result in mutation, cancer or cell death. It is not surprising, then, that both prokaryotic and eukaryotic organisms have evolved mechanisms to repair DNA damaged by alkylation (11). The various base modifications which may arise following exposure of DNA to alkylating agents include: N^1-methyladenine, N^3-methyladenine, N^6-methyladenine, N^7-methyladenine, N^1-methylguanine, N^2-methylguanine, N^3-methylguanine, N^7-methylguanine, N^3-methylcytosine, N^4-methylcytosine, N^3-methylthymine, O^2-methylthymine, O^4-methylthymine, O^2-methylcytosine, and O^6-methylguanine (2). As is readily discerned, the nitrogen atoms on DNA bases are preferred targets. This is due to the fact that the ring nitrogens are much more nucleophilic than the oxygen atoms, with N^3 of adenine and N^7 of guanine being the most reactive (26). This highly nucleophilic character of the nitrogen atoms renders them susceptible to attack by alkylating agents, since such compounds are electrophilic (26). Such agents can be monofunctional (i.e., can interact with single, but varied, nucleophilic centers in DNA) or bifunctional (i.e., can react with two sites in DNA). MMS is an example of a monofunctional agent, while N-methyl N'-nitro nitrosoguanidine (MNNG) is a bifunctional alkylating compound.

Although many of the above base modifications are relatively innocuous, some, such as N^3-methyladenine (N^3meA) and O^6-methylguanine (O^6meG), exhibit a high level of cytotoxicity if not removed from DNA (14). N^3meA is cytotoxic because it presents an impediment to DNA replication (3). In contrast, alkylation of the O^6 position of guanine creates a modified base with the coding properties of adenine (31). Thus, the lesion is directly mutagenic causing G C to A T transitions (29). Therefore, the inability to repair N^3meA and O^6meG markedly increases cellular sensitivity to alkylating agents. This has been shown most dramatically by studying mutants of Escherichia coli deficient in the repair of N^3meA and O^6meG. Such mutants are readily killed after exposure to even low levels of MMS or MNNG.

While much of what is known about alkylation repair has been gleaned from studies utilizing E. coli, higher organisms are also under intense scrutiny (4,30). What is striking is that the mechanisms to repair N^3meA and O^6meG seem to be functionally conserved. More specifically, N^3meA is removed by the action of 3-methyladenine DNA glycosylases, whereas O^6meG is repaired through the action of O^6-methylguanine DNA methyltransferases. DNA glycosylases hydrolyze the bond between the nitrogen atom on an alkylated base and the carbon atom of the deoxyribose sugar (10). This results

in the formation of an apurinic or apyrimidinic (AP) site (10). Subsequent to the action of the glycosylase, AP endonucleases cleave the phophodiester bond at the AP site (10,18). The gap is then filled in and sealed by DNA polymerase I and DNA ligase (10). Rather than liberating the toxic O^6meG base, cells act to repair the base directly. O^6-methylguanine DNA methyltransferases transfer the methyl group from O^6meG to a cysteine residue within the methyltransferase molecule (24). It thus functions as a suicide enzyme inactivator.

In E. coli the O^6-methylguanine DNA methyltransferase is an 18kD molecule encoded by the ada gene (7). The 18kD molecule is a proteolytic fragment of the larger, complete 37kD Ada protein. The ada gene is arranged in an operon with the alkB gene, for which no function has been described (15). The ada gene is also involved in an adaptive response in E. coli (9,13,17). During this adaptive response, cells develop resistance to an alkylating agent after exposure to sublethal concentrations of the compound (9,13). An additional function of the Ada protein (37kD protein) is as a positive regulatory molecule that activates the alkA gene (among other genes) of E. coli once alkylation damage has occured (22,23). The alkA gene encodes one of two 3-methyladenine DNA glycosylases identified in E. coli. The alkA gene product, 3-methyladenine DNA

glycosylase II (TagII), is a 31kD protein. TagII is required for the adaptive response and is a broad spectrum DNA glycosylase (22,23). TagII removes N^3meA, but also releases N^3meG, N^7meG, N^7meA, and other alkylated bases (30). Finally, TagII is not inhibited by free 3-methyladenine, and, when *alkA* is not induced, cells harbor approximately 10 molecules of TagII.

The second 3-methyladenine DNA glycosylase in *E. coli* is termed TagI (3-methyladenine DNA glycosylase I) and is encoded by the *tag* gene (6,28). TagI is a 21kD molecule capable of removing only N^3meA from damaged DNA. TagI, unlike TagII, is inhibited by free 3-methyladenine and *tag* is constitutively expressed (25). Thus, *E. coli* has two 3-methyladenine DNA glycosylases, both of which are required for full resistance to alkylating agents (such as MMS). Mutation in either *tag* or *alkA* causes increased sensitivity to MMS, though an *alkA* mutation is more deleterious (9,12).

There have been reports that 3-methyladenine DNA glycosylases are present in procaryotes besides *E. coli*. For example, a 3-methyladenine DNA glycosylase and a 7-methylguanine DNA glycosylase have been identified in *Micrococcus luteus* (16). Evidence has also been collected for the existence of 3-methyladenine DNA glycosylases in eucaryotes. For instance, DNA

glycosylases specific for N^3meA have been isolated from human lymphoblasts (4,30) and calf thymus (19).

Although DNA glycosylases are necessary for resistance of E. coli to alkylating agents, they are not wholly sufficient. The generalized system of DNA repair termed the SOS response is also required (35). The SOS response is initiated by the multifunctional RecA protein, a 39kD protein encoded by the recA gene (11,34). The hallmark feature of RecA is its ability to potentiate homologous recombination reactions (5). RecA, however, also acts as a protease. It is in the latter capacity that RecA is involved in DNA repair. RecA cleaves LexA repressor protein, thereby allowing expression of the diverse set of genes involved in SOS repair (e.g., those encoding excision-repair enzymes, photorepair enzymes, and cell division inhibitors) (35). The actual signal which induces recA expression is not known, although single-stranded DNA has been implicated. Interestingly, there is also evidence that the presence of 3-methyladenine in genomic DNA serves to induce the SOS response (3). There is, however, no interaction between recA and the tag and alkA genes (8,22,25); that is, TagI and TagII activities are not part of the SOS response.

For E. coli to be resistant to the toxic action of alkylating agents, functional DNA glycosylases and the

SOS reponse must be fully operational. It should be noted that a recA mutation is the most harmful of the single mutations. This fact notwithstanding, a double 3-methyladenine DNA glycosylase mutatation, i.e., tag alkA, results in MMS sensitivity similar to that caused by a recA mutation.

As our laboratory is interested in bacterial genes which control DNA metabolism, we investigated the possible evolutionary conservation of 3-methyladenine DNA glycosylases, as has been done for RecA (5). The organism we chose to study was Serratia marcescens, a little-studied Gram-negative bacterium. This organism had been thought to be a pathogen of insects only. Recently, however, this organism has been reported to be an opportunistic human pathogen and has increasingly been isolated from those suffering from nosocomial infections. S. marcescens is best known, however, for production of the distinctive red pigment prodigiosin and excretion of copius amounts of hydrolytic enzymes. This latter characteristic is of great interest to industrial microbiologists. Studies directed at understanding the molecular biology of this organism have, therefore, increased in recent years. Our efforts have been concentrated in characterizing the genes of S. marcescens involved in DNA repair and recombination.

CHAPTER I

Molecular cloning and characterization of a genetic region from *Serratia marcescens* involved in DNA repair

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Dr. K. Murphy,
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Dear Dr. Murphy,

This letter is to grant permission for use of your article "Molecular cloning and characterisation of a genetic region from Serratia marcescens involved in DNA repair", to be published in Molecular Microbiology, to be used as part of your Ph.D dissertation.

Yours sincerely,

Dr. C. F. Higgins

Molecular Microbiology (1989) 3(2), 249–255

Notes

Molecular cloning and characterization of a genetic region from *Serratia marcescens* involved in DNA repair

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Summary

We report here the molecular isolation of a DNA fragment which encodes Tag-like activity from the Gram-negative bacterium *Serratia marcescens*. A recombinant plasmid encoding Tag-like activity was isolated from a *S. marcescens* plasmid gene library by complementation of an *Escherichia coli tag* mutant, which is deficient in 3-methyladenine DNA glycosylase I. The clone complements *E. coli tag*, *recA*, *alkA*, but not *alkB*, mutants for resistance to the DNA-damaging agent methyl methanesulphonate (MMS). The coding region of the Tag activity, initially isolated on a 6.5kb *Bam*HI fragment, was defined to a 1.8kb *Bgl*II-*Sma*I fragment. Labelling of plasmid-encoded proteins using maxicells revealed that the 1.8kb fragment encodes two proteins of molecular weights 42000 and 16000. Data presented here suggest that the cloned fragment encodes a DNA repair protein(s) that has similar activity to the 3-methyladenine DNA glycosylase I of *E. coli*.

Introduction

DNA-alkylating agents produce lesions which result in mutation, cell death or cancer. Both prokaryotic and eukaryotic organisms have evolved mechanisms which repair DNA damage induced by alkylation (Hanawalt *et al.*, 1979). Also, some prokaryotic cells can become resistant after treatment with sub-lethal doses of alkylating agents (Evensen and Seeberg, 1982; Karran *et al.*, 1982); this is termed the adaptive response. The responses to alkylation damage have been studied most extensively in *Escherichia coli* (Hanawalt *et al.*, 1979). Numerous genes are now known to be involved in the repair of alkylated DNA. The products of these genes include DNA glycosylases, methyltransferases, AP endonucleases, recombinases, and DNA polymerase I (Friedberg, 1985). In the adaptive response, the products of at least two genes, *ada* and *alkA*, are required (Evensen and Seeberg, 1982; Karran *et al.*, 1982). The *ada* gene encodes O^6-methylguanine DNA methyltransferase, which is also a positive regulating element capable of modulating the *E. coli* response to alkylation damage (Evensen and Seeberg, 1982; Lemotte and Walker, 1985; Schendel and Robins, 1978; Teo *et al.*, 1984). The *alkA* gene encodes 3-methyladenine DNA glycosylase II and its expression is induced by Ada protein (Nakabeppu *et al.*, 1984a; Nakabeppu *et al.*, 1984b). While AlkA principally removes 3-methyladenine from the DNA, it also liberates 3-methylguanine, 7-methylguanine, 7-methyladenine and other alkylated bases (Thomas *et al.*, 1982). Another *alk* gene, *alkB*, is also involved in resistance to MMS (Kataoka *et al.*, 1983). In *E. coli*, the *alkB* gene is arranged in an operon with the *ada* gene (Kondo *et al.*, 1986). In contrast to the *alkA* and *ada* gene products, a precise role for AlkB has not been determined. Recent speculation postulates that AlkB may be an oxidoreductase (Kondo *et al.*, 1986). The *recA* gene is also required for MMS resistance (Hanawalt *et al.*, 1979; Walker, 1984). *E. coli recA* mutants are highly sensitive to MMS since induction of the SOS response does not occur in such strains (Hanawalt *et al.*, 1979; Walker, 1984). Not surprisingly, this property has allowed the isolation of *recA* genes from a number of Gram-negative bacteria using complementation of *E. coli recA* strains (Goldberg and Meiklanos, 1986; Koomey and Falkow, 1987).

A second DNA glycosylase, 3-methyladenine DNA glycosylase I, is encoded by the *tag* gene (Clarke *et al.*, 1984; Sakumi *et al.*, 1986). The *tag* gene is constitutively expressed and not subject to regulation by Ada (Riazuddin and Lindahl, 1978). Unlike AlkA, Tag has a much narrower reaction spectrum. In fact, Tag removes only 3-methyladenine from damaged DNA (Thomas *et al.*, 1982). Mutations in either the *tag* or *alkA* genes result in increased levels of sensitivity to the monofunctional alkylating agent MMS, although mutation at *alkA* is more deleterious (Evensen and Seeberg, 1982).

As *E. coli* and higher organisms have been shown to possess the capacity for DNA repair, it is reasonable to assume that other Gram-negative bacteria also have DNA repair mechanisms. Moreover, since *E. coli* and higher organisms (Male *et al.*, 1985; Singer and Brent, 1981) are

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known to depend in part upon DNA glycosylases to effect repair of damaged DNA, we found it interesting to examine other Gram-negative organisms for the presence of such enzymes. Such a study is directed towards determining the possible evolutionary conservation of DNA glycosylases in the bacterial world, as has already been shown for RecA (Clark, 1973). The organism we chose to study was *Serratia marcescens* since our laboratory has worked extensively with this bacterium. We report here the cloning of a *S. marcescens* DNA fragment which can effectively complement *E. coli tag*, *recA* and *alkA* mutants for resistance to MMS. Complementation of an *alkB* mutant was not accomplished. Furthermore, the cloned fragment failed to complement *E. coli ada* mutants for resistance to the multifunctional alkylating agent *N*-methyl-*N*′-nitro-nitrosoguanidine (MNNG). Our data suggest that *S. marcescens* synthesizes a protein which, while functionally similar to Tag of *E. coli*, is sufficiently different to be able to partially complement an *E. coli recA* mutant.

Results

Construction of recombinant plasmids which restore MMS resistance to an E. coli tag mutant

The initial step in this work was to construct plasmids which could confer resistance to MMS upon *E. coli* mutants lacking wild-type levels of 3-methyladenine DNA glycosylase activity. One such mutant, *tag-1*, is deficient in 3-methyladenine DNA glycosylase I (Karran *et al.*, 1980). Thus the strategy was to render an *E. coli tag* mutant resistant to MMS through complementation by a *S. marcescens* DNA glycosylase. Hybrid plasmid molecules were constructed by inserting *Bam*HI fragments of partially digested *S. marcescens* genomic DNA into the *Bam*HI site of pBR322. The plasmid library was used to transform an *E. coli tag* mutant strain BK2114 (Eversen and Seeberg, 1982). Transformants were selected on LA plates containing ampicillin and chlortetracycline. The inclusion of chlortetracycline permitted direct selection of transformants which were tetracycline sensitive because of fragment insertion within the tetracycline resistance gene of pBR322 (Macy and Nunn, 1981). Individual transformants were replica-plated onto LA plates containing ampicillin, chlortetracycline and 0.04% MMS. After incubation for 24 h, two colonies grew on the MMS medium. To ensure that growth was due to heterologous complementation by *Serratia* Tag, plasmid DNA from each isolate was reintroduced into BK2114. All resulting transformants were found to exhibit an MMS-resistant phenotype. Plasmid DNA was cleaved by various endonucleases and analysis of DNA fragment patterns showed that each of the two plasmids contained an identical 6.5 kb *Bam*HI fragment derived from the *S. marcescens* chromosome. One of the plasmids, designated pSM1, was chosen for further study.

Mapping and subcloning of the S. marcescens fragment encoding Tag activity

Restriction mapping of pSM1 was performed, yielding the partial map shown in Fig. 1. To localize the putative *tag* coding region within the cloned DNA, deletion derivatives of the insert were constructed. Figure 1 shows that an internal 3.5 kb *Bgl*II fragment is present in the insert. This permitted the construction of a 3 kb deletion derivative of pSM1. Plasmid pSM1 was digested with *Bgl*II and self-ligated, which resulted in the production of pSM2 (Fig. 1). Plasmid pSM2 was used to transform BK2114 to ampicillin resistance. These transformants were as resistant to MMS as pSM1 transformants were.

Further delimitation of the region containing the Tag activity was accomplished by subcloning the 1.8 kb *Sma*I-*Bgl*II fragment from pSM2 into pUC18 (Fig. 1). This plasmid was designated pSM4. Concomitantly, pSM2 digested with *Sma*I and *Bgl*I was end-filled and religated, yielding pSM3. Both constructs were introduced into BK2114 and resulting transformants were screened for MMS resistance. Only those cells transformed with pSM4 were resistant to MMS, indicating that the activity which complemented for MMS resistance was encoded by a gene(s) which mapped on the 1.8 kb *Sma*I-*Bgl*II fragment. A more detailed restriction map of this fragment was then generated (Fig. 1). Lastly, pSM4 was analysed by insertion and deletion mutagenesis. As depicted in Fig. 1, an APH gene cartridge (which encodes kanamycin resistance), digested with *Eco*RI, was inserted at the *Eco*RV site of the pSM4 insert. Additionally, the 0.8 kb *Sma*I-*Bst*EII fragment was deleted from pSM4. These last two plasmids, designated pSM5 and pSM6, respectively, did not impart MMS resistance to BK2114.

pSM4 complements E. coli tag, recA and alkA but not alkB or ada mutants

It was reasoned that complementation of a mutant *tag* lacking 3-methyladenine DNA glycosylase I was accomplished by a *S. marcescens* DNA glycosylase. This assumption led to the hypothesis that pSM4 might also complement an *E. coli alkA* mutant which lacks 3-methyladenine DNA glycosylase II. This seemed likely since it has been shown that Tag can substitute for AlkA in *E. coli alkA* mutants with no reduction in viability (Kaasen *et al.*, 1986). The hypothesis was tested by introducing pSM4 into an *E. coli alkA* strain. Additionally, the ability of

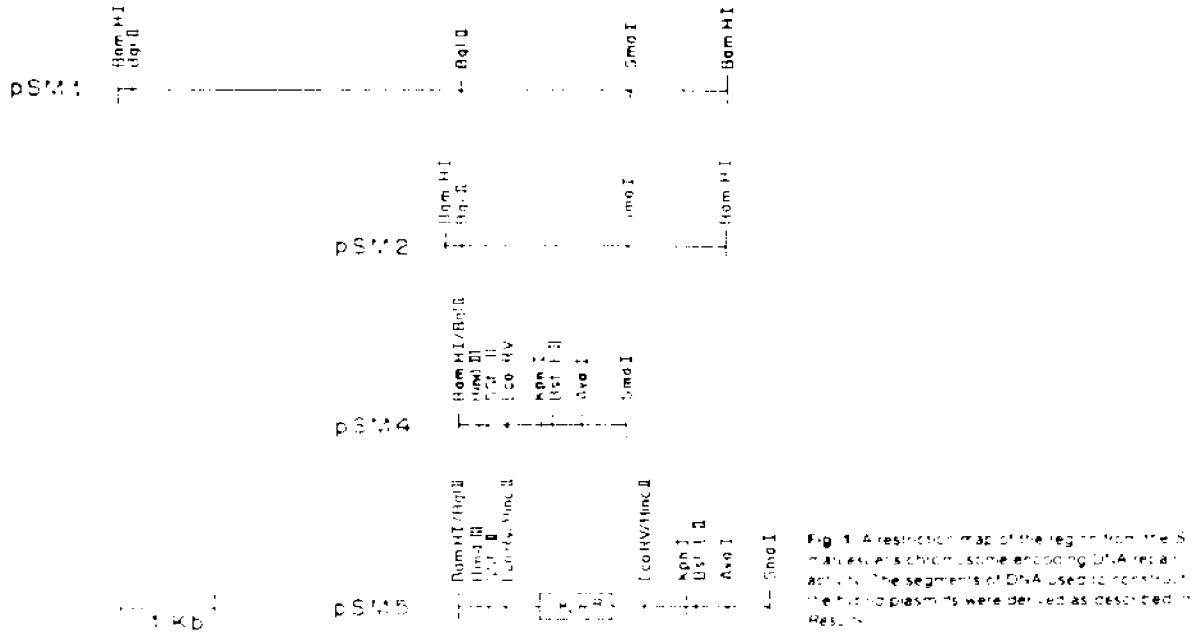

Fig. 1. A restriction map of the region from the *S. marcescens* chromosome encoding DNA repair activity. The segments of DNA used to construct the hybrid plasmids were derived as described in Results.

pSM4 to complement two other *E. coli* repair mutants, *recA* and *alkB*, was examined. Finally, the ability of pSM4 to restore resistance to the mutagenic alkylating agent MNNG in *E. coli ada* mutants was also tested.

E. coli strains used in these complementation studies were BK2114 (*tag*), χ2813 (*recA*), PE353 (*alkA*), MV1601 (*alkB*) and PJ1, PJ3, PJ5, PJ6 (*ada*). The genotype and source of each are listed in Table 2. These *E. coli* strains were transformed with pUC18, pSM4, pGW2607 and pJC859. MV1601 was already ampicillin resistant (Table 2); consequently, a higher concentration of ampicillin (1 mg ml^{-1}) was used to select for the presence of plasmids. Individual isolates from all transformations were picked and plated on MMS plates or MNNG plates (only *ada* mutants were tested on MNNG). Table 1 shows the results of these experiments. Plasmid pSM4 complemented *E. coli tag* and *alkA* mutants for growth on MMS. Unexpectedly, pSM4 also complemented the *E. coli* recA mutant. The MMS sensitive *alkB* mutant, MV1601, was not complemented by pSM4. As expected, pUC18 without insert did not complement any of the repair mutations. Plasmid pGW2607 complemented the *ada*, *alkB* and *tag* mutations. Plasmid pJC859 complemented only strain χ2813, an *E. coli recA* mutant. These data indicated that pSM4 encoded activity which was specific for the repair of MMS-induced DNA lesions. The ability of pGW2607 to complement BK2114 (*tag*) is presumably due to increased expression of the inducible *alkA* gene by the plasmid-encoded Ada protein. This would result in more 3-methyladenine DNA glycosylase II molecules in the cell. Complementation of MV1601 by pGW2607 was, of course, expected. Since the *E. coli recA* strain (χ2813) was complemented by pSM4 there existed the possibility that a *recA*-like gene from *S. marcescens* had been cloned. This possibility was eliminated because the *recA* strain transformed with pSM4 remained highly sensitive to UV light and was incapable of propagating a lambda (*red gam*) bacteriophage.

Characterization of pSM4 complementation

Although the plate tests described above suggested that pSM4 contained a *S. marcescens* DNA fragment which encoded DNA-repair activity, a more quantitative analysis was required. The following experiment (described in *Experimental procedures*) was performed and based on the observation that *E. coli tag*, *alkA* (Evensen and Seeberg, 1982) and *recA* (Owtrimm and Coleman, 1987) mutants exhibit marked sensitivity to MMS in buffer. Survival curves, then, for *E. coli tag*, *recA*, and *alkA* strains harbouring pSM4 or vector alone, were determined. Figure 2 shows the survival curves for *E. coli tag*, *recA* and

Table 1. Complementation of *E. coli* DNA repair mutants

E. coli strain	Plasmid			
	pUC18	pSM4	pUC859	pGW2617
λ2813[a] (*recA*)	-	+	-	
PF353[b] (*alkA*)	-	+		
BK2114[c] (*tag*)	-	+		+
MV1612[b] (*alkB*)				-
PQ3516 (*tag*)				

a. MMS was used at a concentration of 0.02%.
b. MMS was used at a concentration of 0.04%.
c. MNNG was used at a concentration of 0.01%.

alkA strains. The *tag* mutant (BK2114) was complemented by pSM4 such that the survival rate at 120 min was 84% as compared with 4% for the pUC18 control. The observed complementation for the *recA* strain was not as great as that for the *tag* mutant. This fact notwithstanding, significant restoration of MMS-resistance was accomplished by pSM4. Strain λ2813 (*recA*) had a 27% survival rate at 120 min when harbouring pSM4, whereas the pUC18 control exhibited only 2% survival. Figure 2 also shows the results for the *alkA* strain (PF353). Interestingly, this mutant was not complemented in a manner comparable to the *E. coli tag* and *recA* mutants. Plasmid pSM4 did, however, increase the survival of PF353 by three- to five-fold as compared with pUC18. This latter result was somewhat surprising as it was expected that *E. coli alkA* and *tag* mutants would be complemented with similar efficiency by pSM4.

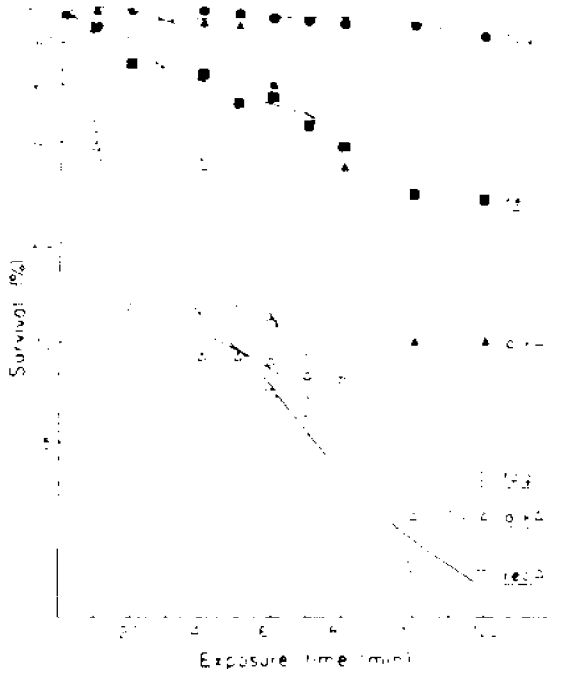

Fig. 2. Survival of *E. coli tag*, *recA* and *alkA* strains harbouring the recombinant plasmid pSM4 or pUC18 after exposure to MMS as described in *Experimental procedures*. Open symbols refer to strains harbouring pUC18 while closed symbols represent strains harbouring pSM4. Symbols: circles, *E. coli tag* (BK2114); triangles, *E. coli alkA* (PF353); squares, *E. coli recA* λ2813.

Identification of the S. marcescens Tag protein

The proteins encoded by plasmids pSM4, pSM5 (present in mutant of pSM4) and pSM6 (deletion mutant of pSM4) were analysed in maxicell experiments (Sancar *et al.*, 1979). Plasmid pSM4 encoded the synthesis of two *S. marcescens* proteins of molecular weights 42,700 and 16,000 (Fig. 3), which were in addition to the vector-encoded β-lactamase. The processed and unprocessed forms have molecular weights of 29,000 and 31,000, respectively. Plasmid pSM5 encoded only the β-lactamase and APH gene product. The deletion construct pSM6 produced the β-lactamase, the 16kD protein and a 24kD protein. The 42kD protein was not produced by pSM6. The 24kD protein probably represents a truncated version of the 42kD protein produced by pSM4. From these data it is evident that the 42kD protein is a fusion protein and both pSM4 and pSM6 synthesize the 16kD protein, although only pSM4 is active. However, the possibility that the 16kD protein is also required but is not by itself sufficient to effect DNA repair has not been ruled out.

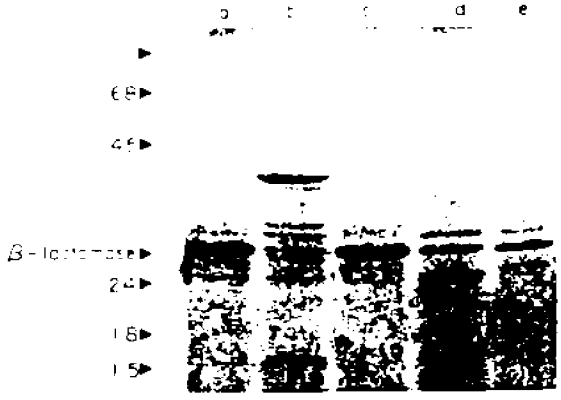

Fig. 3. Autoradiograph of SDS-polyacrylamide gel of proteins synthesized by plasmid-encoded genes in maxicells. Plasmid DNA was isolated from a culture of *E. coli* K12 (DL210) harbouring the plasmid of interest and maxicells were prepared as described by Sancar *et al.* (1979). Ampicillin was added at 100 μg ml⁻¹ to cultures containing pSM4, pSM5 or pSM6. The values to the left of the figure are molecular weights in kilodaltons. The proteins encoded by pSM4, pSM5 and pSM6 are shown on the right. β-lactamase, 31 kD; 24 kD, 16 kD and 15 kD proteins encoded by pSM4 and pSM6. The unprocessed and processed forms of β-lactamase are indicated.

From the restriction map and the protein data it can be concluded that approximately 1580 bp would be required to code for the 42 kD and 16 kD proteins if two distinct genes encode these two proteins. Thus, nearly 88% of the insertion in pSM4 must be coding sequence, if indeed two distinct genes are present in the 1.8 kb insert. The possibility that overlapping genes encode the 42 kD and 16 kD proteins cannot be excluded. Furthermore, it is also possible that the coding regions for these two proteins constitute an operon, as do the *ada-alkB* genes of *E. coli* (Kondo *et al.*, 1986). It is also evident that transcription is initiated at an internal *Serratia* promoter, and not the *lacZ* promoter of pUC18, since no *S. marcescens* proteins were encoded by pSM5. Conversely, both pSM4 and pSM6 encoded *S. marcescens* proteins.

Discussion

We have cloned a segment of the *S. marcescens* genome that encodes a protein which is functionally analogous to the Tag protein of *E. coli*. This conclusion is based on heterologous complementation of an *E. coli tag* mutant. It was also shown that the cloned fragment could complement an *E. coli recA* mutant, but only for MMS resistance. Additionally, the *Serratia* Tag activity complemented an *E. coli alkA* mutant, but not to the same degree as with the *tag* and *recA* mutants. The putative Tag protein differs in size from the Tag protein of *E. coli*. The *Serratia* protein is a 42 kD molecule whereas the *E. coli* Tag protein has a molecular weight of 21,100 (Sakumi *et al.*, 1986).

The ability of the *Serratia* repair activity to complement three distinct *E. coli* repair mutants is interesting. Certain aspects of the complementation data are, however, puzzling. For instance, it was expected that a Tag-like protein which complements the *E. coli tag* mutant so efficiently (Fig. 2) would be just as active in restoring MMS resistance to an *alkA* mutant. It is unclear as to why this did not occur. Full complementation is certainly the case when an *E. coli tag* gene is used to complement an *E. coli alkA* mutant (Kaasen *et al.*, 1986). One reason for the poorer complementation of the *E. coli alkA* mutant by pSM4 might be that other alkylated bases which cannot be removed by the *Serratia* protein in an *E. coli alkA* strain cause cell death. If, however, *E. coli* and *S. marcescens* repair alkylated DNA by similar mechanisms then this possibility seems unlikely, in light of the report cited previously (Kaasen *et al.*, 1986). The fact that an *E. coli recA* strain is complemented by pSM4 presents a further complication. A possible explanation is that the cloned *Serratia* fragment encodes a glycosylase which is present at elevated levels because its coding region is on the high-copy vector, pUC18. This protein, along with the two endogenous 3-methyladenine DNA glycosylases, Tag and AlkA, may be sufficient to excise enough alkylated bases to restore, in part, resistance to MMS even in the absence of the SOS response. An intriguing experiment would be to determine whether the *tag* or *alkA* genes of *E. coli* could complement an *E. coli recA* strain, as does the cloned *Serratia* fragment.

The inability of pSM4 to complement an *E. coli alkB* mutant is more readily explained. Presently, the role of AlkB is not known, although it is required for resistance to MMS by *E. coli* (Kataoka and Sekiguchi, 1985; Kataoka *et al.*, 1983). The observation that pSM4 complemented *E. coli tag, recA, alkA*, but not *alkB* mutants, supports the idea that AlkB does not act to liberate alkylated bases directly (Kondo *et al.*, 1986). AlkB does however participate at some step(s) in the repair of alkylated DNA (Kataoka *et al.*, 1983). It may act in a capacity which augments or allows excision of alkylated bases by DNA glycosylases. If, in fact, AlkB is an oxidoreductase (Kondo *et al.*, 1986) it may function to convert alkylated bases to more innocuous forms. Therefore, since our complementation data indicate that pSM4 codes for a *Serratia* protein which acts to repair DNA directly, it is not surprising that the *E. coli alkB* mutant was not complemented by pSM4. The data also showed that the *E. coli tag* mutant could be complemented by pGW2607. This result is probably caused by an increased level of 3-methyladenine DNA glycosylase II (i.e. AlkA) within the cell. An increased level could be caused by overproduction of Ada from pGW2607, which would serve to induce *alkA* expression at a higher than normal level.

The protein data correlate well with the complementation data. As shown in Fig. 3, pSM4 codes for 42 kD and 16 kD proteins, pSM5 encodes no *Serratia* proteins and pSM6 encodes a 24 kD protein and the 16 kD protein. Only pSM4 has MMS resistance activity. These data strongly suggest that the 42 kD protein is the molecule actively involved in the repair of alkylated DNA in the various *E. coli* repair mutants. While the data indicate that the 42 kD protein is the active molecule, the 16 kD protein may have an auxiliary role. Future experiments which will utilize constructs coding only for the 42 kD protein will determine if the 16 kD is actually required for DNA repair. If the active protein is the 42 kD protein, this implies a significant difference between the Tag protein of *E. coli*, a 21 kD molecule, and the Tag protein of *S. marcescens* even though pSM4 was highly effective in restoring MMS resistance to the *E. coli tag* mutant.

The question of whether or not one gene encodes the observed DNA repair activity is currently being addressed. The fact that pSM4 synthesizes two *Serratia* proteins precludes the conclusion that a single DNA repair gene from *S. marcescens* has been cloned and is solely sufficient for complementation of the *E. coli* repair mutants.

Finally, further work will examine the exact biochemical

role of the *Serratia* protein. More specifically, the question of the actual repair mechanism must be analysed. The fact that the cloned fragment complements a 3-methyladenine DNA glycosylase I mutant (*tag*) so efficiently is excellent preliminary evidence that the cloned fragment encodes a DNA glycosylase from *S. marcescens*. The possibility that the *Serratia* repair protein represents a novel repair molecule must also be explored since pSM4 complemented both the 3-methyladenine DNA glycosylase mutants and the *recA* mutant.

Experimental procedures

Chemical reagents and enzymes

Methyl methanesulphonate (MMS) and *N*-methyl *N*'-nitroso-guanidine (MNNG) were purchased from the Aldrich Chemical Company. Restriction endonucleases, T4 DNA ligase, DNA polymerase I and DNA polymerase I large fragment (Klenow fragment) were purchased from Bethesda Research Laboratories. L-[^{35}S]-methionine was purchased from New England Nuclear.

Bacterial strains

All bacterial strains and the source of each are listed in Table 2.

Table 2. Bacterial strains.

Strain	Relevant features	Source
Serratia marcescens		
ATCC 25419	wild type	Laboratory stock
E. coli K-12		
AB1157	*recA56*	NEB[a]
PR353	*alkA1*	P. Foster
EK2714	*tag2*	M. Volkert
UV1601	*alkB* Mud(Ap^R *lac*)	M. Volkert
PJ1356	*alkA1356*	G. Walker
CSR603	*recA1 uvrA6 phr-1*	B. Bachmann

[a] New England Biolabs.

Plasmids

Plasmids used as cloning vectors were pBR322 and pUC18. Hybrid plasmids were designated as pSM constructs. The individual plasmids and cloning strategies are depicted in Fig. 1. Plasmid pGW2607 was a kind gift from Dr Graham C. Walker and contains the *E. coli alkA-alkB* operon in pBR322 (Lemotte and Walker, 1985). Plasmid pJC659 was kindly supplied by Dr A. J. Clark and contains the *E. coli recA* gene in pBR322.

Media

Luria broth (Miller, 1972), chlortetracycline medium (Maloy and Nunn, 1981) and minimal salts medium less glucose (Manatis *et al.*, 1982) were as described. Antibiotics were used at the following concentrations: ampicillin 50 μg ml^{-1}, tetracycline 10 μg ml^{-1}, kanamycin 50 μg ml^{-1} and chlortetracycline 50 μg ml^{-1}.

MMS sensitivity

Individual *E. coli* strains were tested for MMS sensitivity by streaking on Luria agar (LA) plates supplemented with MMS at concentrations varying from 0.01% to 0.05%. Sensitivity of repair mutants harbouring the recombinant plasmid or vector only was determined as follows: exponentially growing cultures were harvested by centrifugation and resuspended in minimal salts buffer (30°C). MMS was added to a final concentration of 0.03%. Cultures were incubated at 30°C and aliquots were removed at defined intervals, diluted, and plated on LA plates containing ampicillin at the appropriate concentration. After growth at 30°C for 18 h, colonies were counted and survival curves were derived.

Genetic manipulations and recombinant DNA techniques

Genetic manipulations were performed as described (Maniatis *et al.*, 1982).

Identification of plasmid-encoded proteins in maxicells

Hybrid plasmids were used to transform *E. coli* strain CSR603. Plasmid derived proteins were labelled with L-[^{35}S]-methionine using the maxicell technique (Sancar *et al.*, 1979) and resolved by electrophoresis on sodium dodecyl sulphate-polyacrylamide gels.

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CHAPTER II

Evidence for unique DNA repair activity encoded by a cloned *Serratia marcescens* gene: Suppression of *Escherichia coli* mutations which reduce repair of alkylated DNA

Kirk Jensen, Director
ASM Publications Department
1913 I Street, N.W.
Washington, D.C. 20006

June 5, 1989

Dear Mr. Jensen:

I am writing to request permission to include a preprint of my J. Bacteriol. publication in my doctoral dissertation. The article will appear in the September 1989 issue and is entitled: "Evidence for Unique DNA Repair Activity Encoded by a Cloned Serratia marcescens Gene: Suppression of Escherichia coli Mutations that Reduce Repair of Alkylated DNA".

A prompt reply would be greatly appreciated. Thank you very much Mr. Jensen.

Most sincerely,

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PERMISSION GRANTED PENDING AUTHOR PERMISSION
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Evidence for Unique DNA Repair Activity Encoded by a Cloned *Serratia marcescens* Gene: Suppression of *Escherichia coli* Mutations That Reduce Repair of Alkylated DNA

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A recombinant plasmid containing a *Serratia marcescens* DNA repair gene has been analyzed biochemically and genetically in *Escherichia coli* mutants deficient for repair of alkylated DNA. The cloned gene suppressed sensitivity to methyl methanesulfonate of an *E. coli* strain deficient in 3-methyladenine DNA glycosylases I and II (i.e., *E. coli tag alkA*) and two different *E. coli recA* mutants. Attempts to suppress the methyl methanesulfonate sensitivity of the *E. coli recA* mutant by using the cloned *E. coli tag* and *alkA* genes were not successful. Southern blot analysis did not reveal any homology between the *S. marcescens* gene and various known *E. coli* DNA repair genes. Biochemical analysis with the *S. marcescens* gene showed that the encoded DNA repair protein liberated 3-methyladenine from alkylated DNA, indicating that the DNA repair molecule is an *S. marcescens* 3-methyladenine DNA glycosylase. The ability to suppress both types of *E. coli* DNA repair mutations, however, suggests that the *S. marcescens* gene is a unique bacterial DNA repair gene.

When DNA is exposed to the monofunctional alkylating agent methyl methanesulfonate (MMS), N^3-methyladenine is one of the major products formed (2). Excision of this base is accomplished by 3-methyladenine DNA glycosylases, which exist in both procaryotic and eucaryotic organisms (6). Base excision by this type of enzyme results in liberation of the modified base in its free form (11, 13, 15, 25).

It has long been known that procaryotic organisms lacking the capacity to repair N^3-methyladenine are more sensitive to alkylation damage than are organisms which have such repair mechanisms (20). For example, it has been shown that *Escherichia coli* mutants deficient in 3-methyladenine DNA glycosylases I and II (i.e., *E. coli tag alkA*) are highly sensitive to DNA alkylation, resulting in the formation of 3-methyladenine (3, 5). This observation provided strong evidence that 3-methyladenine has cytotoxic effects if not removed from DNA. Furthermore, such results stimulated extensive genetic and biochemical studies, which have culminated in the cloning of 3-methyladenine DNA glycosylase genes from *E. coli* and in the elucidation of the biochemical function of two such enzymes, TagI (3-methyladenine glycosylase I) and TagII (3-methyladenine glycosylase II) (5, 8, 9, 18, 19, 21, 23, 27).

In addition to functional DNA glycosylases, *E. coli* is dependent on other cellular responses for resistance to alkylating agents such as MMS. The generalized DNA repair cascade known as the SOS response is also required (29). This response is initiated by cleavage of the LexA repressor protein by the *recA* gene product (29). Thus, *E. coli recA* mutants exhibit extreme sensitivity to alkylating agents (e.g., MMS) since the SOS response cannot be elicited (6, 28). While it is required for resistance to alkylation damage, the SOS repair pathway does not regulate the activity of TagI or TagII in *E. coli*. In fact, induction of *alkA* was shown to be independent of *recA* (4, 28), and *tag* is expressed constitutively (21).

We have been studying DNA repair in gram-negative bacteria other than *E. coli* in an effort to determine whether certain DNA repair mechanisms have been conserved through evolution. Our efforts have focused on the isolation and characterization of a DNA glycosylase gene(s) from *Serratia marcescens*. The initial paper in this study described the suppression of MMS sensitivity exhibited by *E. coli tag*, *alkA*, and *recA* mutants by a cloned *S. marcescens* DNA repair gene (17), which was preliminarily characterized as a functional analog of *E. coli tag*.

In the current report we present evidence that the *S. marcescens* DNA repair gene is unique in comparison to the *E. coli tag*, *alkA*, and *recA* genes. More specifically, only the *S. marcescens* gene was capable of suppressing *E. coli tag alkA* and *E. coli recA* mutations. The active protein encoded by the cloned gene, a 42-kilodalton (kDa) molecule (17), releases 3-methyladenine from alkylated substrate DNA. Finally, Southern blot analysis failed to reveal any homology between the *S. marcescens* gene and various *E. coli* genes known to be involved in DNA repair. Our results suggest that *S. marcescens* possesses a novel DNA repair mechanism which is functional in *E. coli* and can effectively suppress distinct mutations which result in deficiency for the repair of alkylated DNA.

Suppression of *E. coli tag alkA* and *E. coli recA* mutations. In our previous report, it was speculated that a possible explanation for suppression of the *E. coli recA* mutation by the *S. marcescens* gene (*rpr*) was that the cloned gene encoded a 3-methyladenine DNA glycosylase that restored, in part, resistance to MMS. It was reasoned that if this were true, the cloned *E. coli tag* and *alkA* genes might also be able to suppress an *E. coli recA* mutation. To determine the validity of this hypothesis, the comparative abilities of the *rpr* and the *E. coli tag* and *alkA* genes to suppress an *E. coli recA* mutation and an *E. coli tag alkA* mutation were determined.

The recombinant plasmids used in this experiment are listed in Table 1. Plasmid pSM9 harbors the *rpr* gene on a 2.5-kilobase (kb) *SmaI-HindIII* fragment. This plasmid is a deletion derivative of pSM4, which has been described

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or phenotype[a]	Source or reference
S. marcescens ATCC 25419	Wild type	Laboratory stock
E. coli K-12		
χ2813	*recA56*	NEB[b]
MV1932	*tag-1 alkA1*	M. Volkert
DH5α	*recA1*	Laboratory stock
PF1018	*alkA1 recA1 tag*$^+$	
Plasmids		
pCY5	*tag*$^+$ Amp^r	M. Sekiguchi
pYN1000	*alkA*$^+$ Amp^r Tet^r	M. Sekiguchi
pJC859	*recA*$^+$ Amp^r	A. J. Clark
pSM9	*rpr*$^+$ Amp^r	This work
pUC18	Amp^r	Laboratory stock
pGW260[c]	*ada*$^+$ *alkB*$^+$ Amp^r	G. Walker

[a] *rpr* is the *S. marcescens* DNA repair gene.
[b] NEB, New England BioLabs.

before (17). Plasmid pSM9 is as effective at restoring MMS resistance as pSM4. Both plasmids produce the same *S. marcescens* proteins (i.e., polypeptides of 42 and 16 kDa). Furthermore, analysis of deletion derivatives of pSM9 revealed that it was not possible for the cloned segment to contain two distinct, nonoverlapping genes (data not shown). Therefore, the 16-kDa protein is either a breakdown product of the 42-kDa protein or the product of a gene having a reading frame overlapping that of the 42-kDa-protein gene.

Plasmids pSM9, pCY5, *tag*$^+$ in pUC8 [24]), pYN1000 (*alkA*$^+$ in pBR322 [19]), pJC859 (*recA*$^+$ in pBR322), and pUC18 were introduced into *E. coli* strains MV1932 (*tag alkA*) and χ2813 (*recA*). Table 2 shows the plating efficiencies of these two strains harboring the various plasmids. This experiment was performed as described previously (5). pCY5 (*tag*$^+$) and pYN1000 (*alkA*$^+$) did not restore MMS resistance to the *E. coli recA* mutant (χ2813). Also, pJC859 (*recA*$^+$) did not restore resistance to MV1932 (*E. coli tag alkA*). Plasmid pSM9, however, significantly increased the MMS resistance of the *E. coli tag alkA* mutant (MV1932) and the *E. coli recA* mutant (χ2813). Although pSM9 was not completely effective in either the *tag alkA* or *recA* mutant, it is clear that the cloned *S. marcescens* gene was capable of suppressing the MMS sensitivity caused by the two distinct *E. coli* DNA repair mutations.

There existed the possibility that the effect of pSM9 on *recA* (χ2813) was allele specific. To alleviate this concern, a second *E. coli recA* mutant was tested. Strain DH5α carries a *recA1* point mutation, which is distinct from the *recA56* allele of χ2813. As was seen with χ2813, only pJC859 and pSM9 were able to restore MMS resistance to DH5α, proving that the effect of pSM9 was specific for the *recA* mutations and not the individual allele (data not shown). These data indicated that the ability of the *S. marcescens rpr* gene to suppress the *E. coli recA* and *tag alkA* mutations is novel, since none of the *E. coli* DNA repair genes showed such heterologous suppression abilities.

TABLE 2. Effect of various plasmids on the MMS sensitivity of *E. coli* mutants deficient in alkylation repair

Strain and plasmid	Plating efficiency (%) on LB agar containing ampicillin (50 μg/ml) and 0 (control) or 0.5 mM MMS
MV1932(pYN1000)	97.5
MV1932(pCY5)	63.2
MV1932(pSM9)	16.5
MV1932(pJC859)	0.1
MV1932(pUC18)	0.15
χ2813(pYN1000)	0.12
χ2813(pCY5)	0.1
χ2813(pSM9)	1.6
χ2813(pJC859)	56
χ2813(pUC18)	0.1

[a] MV1932 is an *E. coli tag alkA* mutant; χ2813 is an *E. coli recA* mutant.

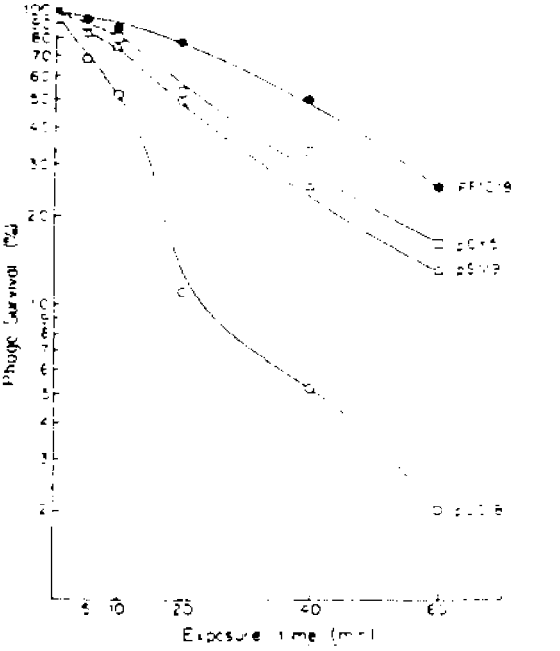

FIG. 1. Effects of pSM9 on the host cell reactivation of MMS-treated bacteriophage lambda cI857 in strain MV1932 (*E. coli tag alkA*). Symbols: □, pUC18; △, pSM9; ○, pCY5; ●, host cell reactivation in strain PF1018 (*tag*$^+$ *alkA*$^+$ *recA*$^+$) without plasmid. MV1932 and PF1018 were derived from parental strain AB1157 (2). MMS was used at a concentration of 50 mM.

Host cell reactivation of MMS-treated bacteriophage. *E. coli* MV1932 is deficient for 3-methyladenine DNA glycosylases I and II. Because it lacks these two enzymes, MV1932 is unable to reactivate bacteriophage lambda which has been exposed to MMS (5, 10). We examined whether pSM9 could restore viability to MMS-treated lambda phage. Figure 1 shows the results of an experiment designed to test this possibility. As expected, lambda was not reactivated by

TABLE 3. Summary of Southern blot hybridization experiments

Sample DNA	Hybridization with gene probes			
	E. coli tag	*E. coli alkA*	*E. coli recA*	*S. marcescens rpr*
S. marcescens	-	-	-	+
E. coli K-12	+	+	+	-
E. coli tag	+	-	ND	-
E. coli alkA	-	+	ND	-
E. coli recA	ND	ND	+	-
E. coli ada alkB	ND	ND	ND	-
S. marcescens rpr	-	-	-	+

[a] A plus sign indicates significant hybridization between probe and sample. A minus sign indicates no hybridization even after extensive exposure of autoradiographic film. A plus minus sign indicates weak hybridization. ND, Not done. Blots were washed under mid stringency conditions.

TABLE 4. 3-Methyladenine DNA glycosylase activity in an *E. coli tag alkA* mutant (MV1932) transformed with various plasmids

Strain and plasmid	N^3-Methyladenine released[a] (fmol)
MV1932	6.0
MV1932(pUC18)	6.0
MV1932(pCY5 *tag*)	25.0
MV1932(pSM9 *rpr*)	23.0
Blank	6.0

[a] 45 fmol of 3-methyladenine were present in the alkylated substrate DNA. The assay for DNA glycosylase activity was as described in Materials and Methods. Equal amounts (32 μg) of cell extract protein were reacted with alkylated substrate DNA. The reaction mixture blank contained an equivalent amount of acetylated bovine serum albumin.

pUC18-containing MV1932. There was a dramatic increase in lambda survival when either pSM9 or pCY5 (*tag*$^+$) was present in MV1932. Neither plasmid, however, restored lambda survival levels to that of *E. coli* PE1028 (*tag*$^+$ *alkA*$^+$).

This assay (22) provided a useful mechanism for evaluating DNA repair activity. The *rpr* gene product was capable of reactivating lambda (Fig. 1). Interestingly, *rpr* was as effective as *tag* in this assay. This result would not be predicted from the data shown Table 2. Why *rpr* was more efficient at host cell reactivation than at restoration of MMS resistance to the *E. coli tag alkA* mutant is unclear. Regardless, this experiment proved that Rpr repairs DNA directly and does not function by preventing damage to DNA.

Sequence homology between the *S. marcescens* DNA repair gene and *E. coli* DNA repair genes. To further the molecular analysis of *rpr*, we tested for the possibility of DNA homology between the *S. marcescens* gene and some known *E. coli* DNA repair genes. The cloned *E. coli* genes used in this experiment included the *recA* gene (pJCS559), *tag* gene (pCY5), *alkA* gene (pYN2000), and the *ada-alkB* operon (pGW2603, a pBR322 derivative containing the *ada-alkB* genes [12]). Also, chromosomal DNA from *E. coli* K-12 was examined. In a Southern blot analysis (26), the above samples were digested with the appropriate restriction endonucleases and probed with the 1.5-kb *Sma*I-*Hin*dIII insert fragment of pSM9. Hybridizations and washings (mid stringency conditions) were carried out as described before (26). Even after extensive exposure of the film, no hybridization of the *S. marcescens* probe to the cloned *E. coli* genes was detected. There was, however, a weak band visible with the *E. coli* chromosomal DNA sample. More specifically, an 8- to 9-kb *Bam*HI *E. coli* chromosomal fragment showed slight hybridization to the probe (data not shown). In a separate experiment, it was shown that an 8-kb *Bam*HI *S. marcescens* fragment hybridized strongly to an *E. coli recA* probe, implying that *S. marcescens* has a *recA* analog. Unpublished observations. Thus, Southern blot analysis showed that the cloned gene had no detectable homology with the *E. coli recA*, *tag*, or *alkA* genes even though it suppressed the MMS sensitivity of *E. coli* mutants defective for those genes. Finally, the *alkA* and *tag* genes were used to probe a *Bam*HI digest of *S. marcescens* chromosomal DNA. No hybridization was detected. The results of Southern blot experiments are summarized in Table 3.

Assay for enzyme activity of pSM9. It was important to characterize the biochemical activity of pSM9 once we had shown its unique capabilities. Therefore, an experiment to determine the ability of the *S. marcescens* DNA repair protein to excise modified bases from alkylated substrate DNA was performed. Extracts from strain MV1932 (*E. coli tag alkA*) harboring the various plasmids were prepared as described before (24). These crude extracts were examined for the ability to remove 3-methyladenine from alkylated substrate DNA. The assay for 3-methyladenine DNA glycosylase activity was done as described before (22). Extracts of cells containing either pCY5 or pSM9 released comparable amounts of 3-methyladenine from alkylated substrate DNA (Table 4). Cell extracts harboring the cloning vector pUC18 as well as extracts from host strain MV1932 did not liberate 3-methyladenine from substrate DNA.

The possibility that pSM9 might have activity towards other DNA base modifications was also examined. From this analysis, it was determined that pSM9 did not encode a protein which possessed the capacity to remove N^1-methyladenine, O^6-methylguanine, N^3-methylguanine, or N^6-methyladenine (data not shown). N^7-methylguanine was released as <5% of the total N^7-methylguanine in the substrate DNA in all samples and thus did not affect the interpretation of the results. These data indicate that suppression of the *E. coli tag alkA* mutation was due to the activity of *S. marcescens* 3-methyladenine DNA glycosylase, i.e., Rpr. Furthermore, Rpr had the same narrow activity spectrum as *E. coli* TagI with respect to removal of alkylated purines (Table 4 and text). Finally, the data in Table 4 are consistent with data shown in Fig. 1. That is, *tag* and *rpr* exhibited similar profiles in host cell reactivation and 3-methyladenine DNA glycosylase activity.

Obviously, the data presented here are somewhat paradoxical. In Table 4, data indicate that the *rpr* gene encodes a protein with 3-methyladenine DNA glycosylase activity, which explains *rpr* suppression of the *E. coli tag alkA* mutation (Table 2 and Fig. 1). One of the most interesting aspects of this work, however, is that *rpr* suppresses the *recA* mutation as efficiently as it does the *tag alkA* mutation. The fact that *rpr* suppresses the *recA* mutation suggests that *rpr* must encode a unique DNA repair activity. One possibility is that Rpr has a lambda *gam*-like function and thus inhibits DNA degradation by RecBCD. Such activity might permit growth of the *E. coli recA* mutants on MMS. Finally, it is known that N^7-methylguanine and N^3-methyladenine are removed by DNA glycosylase activity, with the result being apurinic apyrimidinic (AP) sites left in the DNA (23). Such sites are potentially mutagenic. It is possible that one mechanism to repair AP sites generated subsequent to the action of DNA glycosylase activity involves encoding proteins which have DNA glycosylase activity and AP endonuclease activity residing in the same protein. This type of activity has been found in the T4 bacteriophage (23) and

Micrococcus luteus (7) pyrimidine dimer DNA glycosylases. Perhaps *S. marcescens* has evolved a similar system for the repair of alkylated DNA. It is not possible to determine this at present. The repair protein will have to be purified before we can determine whether or not *rpr* does encode such dual activity.

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CHAPTER III

Sensitization to methyl methanesulfonate of *Escherichia coli* wild type, *xth*, and *nfo* strains by *Serratia marcescens* *rpr* gene

SUMMARY. It is reported here that the *rpr* DNA repair gene of *Serratia marcescens* does not complement an *Escherichia coli xth nfo* AP endonuclease mutation for resistance to methyl methanesulfonate (MMS). Rather, *rpr* sensitized *E. coli* wild type, *xth*, and *nfo* strains to MMS. Also, it was found that *rpr* could not complement a triple *tag alkA recA* mutation in *E. coli*, indicating that there exist limits on *rpr* complementation capabilities. It was determined that *rpr* gene dosage was not a factor in *recA* complementation. MMS sensitization of an *E. coli* wild type strain, however, was directly related to *rpr* copy number. These data indicate that Rpr does not have an associated AP endonuclease activity, nor is it capable of substituting for Tag I, Tag II, and RecA in a *tag alkA recA* background.

INTRODUCTION

When DNA is exposed to alkylating agents various base modifications may arise (Beranek et al., 1980). One such modification, N^3-methyladenine, is produced following treatment of DNA with methyl methanesulfonate (MMS) (Beranek et al., 1980). 3-methyladenine is a cytotoxic lesion because it blocks DNA replication (Boiteux et al., 1984). Thus, to ensure survival organisms have evolved mechanisms to repair 3-methyladenine (Hanawalt et al., 1979; Singer and Brent, 1981). For example, in E. coli removal and repair of 3-methyladenine is effected by the action of 3-methyladenine DNA glycosylases I and II (i.e., Tag I and Tag II) (Clarke et al., 1984; Evensen and Seeberg, 1982; Kaasen et al., 1986; Karran et al., 1982; Karran et al., 1980; Nakabeppu et al., 1984a; Nakabeppu et al., 1984b; Riazuddin and Lindahl, 1978; Sakumi et al., 1986; Thomas et al., 1982) and apurinic/apyrimidinic (AP) endonucleases (Lindahl, 1979; Lindahl, 1982; Friedberg, 1985). AP sites are generated following the liberation of an alkylated base by Tag I or Tag II (Friedberg, 1985). AP sites are highly mutagenic since purines are often improperly inserted by DNA polymerases (Loeb, 1985). The major AP endonuclease in E. coli is exonuclease III (Weiss, 1976; Yajko and Weiss, 1975). This enzyme is encoded by the xth gene

and represents 90% of toal AP endonuclease activity in E. coli (Weiss, 1976; Yajko and Weiss, 1975). Surprisingly, mutation in the xth gene only slightly increases sensitvity to MMS (Ljungquist et al., 1976: Yajko and Weiss, 1975). This is due to the activity of a second AP endonuclease, endonuclease IV, which is encoded by the nfo gene (Cunningham et al., 1986). This enzyme accounts for no more than 10% of total AP endonucleolytic activity in E. coli (Ljungquist et al., 1976). Mutation in nfo does not increase MMS sensitvity significantly (Cunningham et al., 1986). A double mutation (i.e., xth nfo), however, results in markedly increased MMS sensitivity (Cunningham et al., 1986).

We recently reported the cloning and characterization of a DNA repair gene (rpr) from S. marcescens. The rpr gene complements E. coli tag alkA and recA mutations for MMS resistance (Murphy et al., 1989). It was determined that complementation of the tag alkA mutation is due to rpr synthesis of a 42kD protein with 3-methyladenine DNA glycosylase activity and a reaction spectrum identical to that of E. coli Tag I (Murphy et al., 1989). To date, we have not been able to define the biochemical mechanism by which rpr complements the recA mutation. We recently hypothesized that Rpr may have an associated AP endonuclease activity, which might obviate the necessity for RecA

function (Murphy *et al.*, 1989) It has been shown that T4 bacteriophage (Radany and Friedberg, 1980) and *Micrococcus luteus* (Haseltine *et al.*, 1980) encode single proteins which have both DNA glycosylase and AP endonuclease activities.

The object of this study, then, was to determine the extent of *rpr* complementation capabilities. To this end, we tested *rpr* for the ability to complement *E. coli xth*, *nfo*, and *xth nfo* mutations. We also examined the possibility that *rpr* might complement an *E. coli tag alkA recA* mutation. It is reported here that *rpr* cannot complement any of the aforementioned mutations. In fact, *rpr* sensitized *E. coli xth*, *nfo* and wild type strains to the toxic action of MMS.

RESULTS

Sensitization of *E. coli xth* and *nfo* mutants by *rpr*. As *rpr* complemented the *tag alkA* and *recA* mutations with similar efficiency, it was thought that *rpr* must encode a second activity in addition to its 3-methyladenine DNA glycosylase function (Murphy *et al.*, 1989). One possibility was that Rpr also acts as an AP endonuclease. To test this idea we examined *rpr* for the ability to complement *E. coli* AP endonuclease mutations. Strains utilized in this experiment are shown in Table 5. Three plasmids (Table 5) pUC18, pSM4 (*rpr*$^+$ in pUC18) and pCY5 (Sakumi *et al.*, 1986) (*tag*$^+$ in pUC18) were introduced into PF11, PF111, PF1316 and PF1317. This experiment revealed that pSM4 did not complement the *xth nfo* mutation in strain PF1317 (Table 1). Interestingly, pSM4 greatly sensitized the other three strains to MMS. It was also shown that pCY5 sensitized the *xth* mutant to MMS. These data indicated that *rpr* does not encode AP endonuclease activity which allows growth of the *E. coli xth nfo* mutant on MMS. Additionally, these data suggested that Rpr is harmful to wild type and AP endonuclease-deficient strains of *E. coli*.

***E. coli tag alkA recA* mutation is not complemented by *rpr*.** Complementation of *E. coli tag alkA* and *recA* mutations by *rpr* suggested that *rpr* might also restore

MMS resistance to a strain harboring a triple mutation, i.e., tag alkA recA. Strain MV2153 (Table 5) has such a mutation and we tested whether rpr could complement this strain for MMS resistance. Constructs utilized to address this question are listed in Table 5 and described in Experimental procedures. As Table 2 shows, none of the plasmids were capable of complementing the tag alkA recA mutation. These data suggest that rpr cannot compensate for both the lack of 3-methyladenine DNA glycosylase activity and the absence of RecA.

Effect of copy number on rpr complementation of a recA mutation. It was possible that gene dosage might influence the observed complementation of the recA mutation by rpr (Murphy and Braymer, 1989; Murphy et al., 1989). Therefore, the 2.8kb Bam HI-Bgl II fragment from plasmid pSM2 (Murphy and Braymer, 1989) was cloned into pACYC184 yielding pSM11. It should be noted that pSM2 and pSM11 carry S. marcescens genomic DNA in addition to the 1.8kb Bgl II-Sma I rpr insertion in pSM4. It was previously shown, however, that DNA adjacent to the rpr gene did not impart MMS resistance (Murphy and Braymer, 1989). Thus, this was not expected to be a factor in the experiment. Also used to transform the E. coli recA strain (χ2813) were pCM1 (recA$^+$ in pACYC184) and pJC859 (recA$^+$ in pBR322). Table 3 shows that pSM2, pSM4, and pSM11 exhibited similar levels of complementation of the recA mutation in

2813. It is evident that gene dosage does not affect, positively or negatively, the ability of *rpr* to complement the *E. coli recA* mutation.

Sensitization of an *E. coli* wild type strain by *rpr*. As Table 1 shows, *rpr* (pSM4) greatly increased the MMS sensitivity of wild type strain PF11. This phenomenon has also been reported to be induced by clones of the *E. coli alkA* gene, depending on the plasmid construct employed (Kaasen *et al.*, 1986). This fact necessitated an anaylsis of gene dosage effects on sensitization of *E. coli* wild type strains. The following plasmids were used to transform strain PF1018 (Table 5): pUC18, pBR322, pACYC184, pSM2, pSM4, pSM11, pCY5, and pYN1000 (Nakabeppu *et al.*, 1984a) (*alkA*$^+$ in pBR322). Table 4 clearly shows that the level of MMS sensitization by *rpr* is directly related to plasmid copy number. More specifically, pSM11 did not sensitize PF1018 while pSM2 and pSM4 did increase MMS sensitivity. This effect was most pronounced in pSM4 transformants. These results allow the conclusion that overproduction of Rpr is deleterious to the wild type cell.

DISCUSSION

We have presented data indicating that the *S. marcescens rpr* gene does not encode AP endonuclease activity which, if present, should permit growth of an *E. coli xth nfo* mutant on MMS. Furthermore, it was

shown that rpr is actually detrimental to E. coli xth, nfo and wild type strains. It was also demonstrated that rpr cannot complement an E. coli tag alkA recA mutation for MMS resistance. Finally, it was shown that the level of MMS sensitization of E. coli strain PF1018 (wild type) is a function of rpr copy number.

The inability of rpr to complement the three AP endonuclease mutations was not wholly unexpected. What is surprising, though, is that rpr rendered the xth and nfo mutants much more sensitive to MMS (Table 1). Normally, such mutants are quite resistant to MMS (Cunningham et al., 1986; Yajko and Weiss, 1975). Sensitization of the xth mutant can be ascribed to excess 3-methyladenine DNA glycosylase activity since pCY5 (tag^+) exerted the same effect as rpr. This result can be explained. Increased 3-methyladenine DNA glycosylase activity in an xth strain might result in a greater number of AP sites arising which then remain unrepaired. Since exonuclease III represents 90% of the total cellular AP endonuclease activity, the xth mutant would be subjected to MMS mutagenesis and killing if more AP sites are created during a defined time interval than can be correctly repaired. In contrast, it is not readily evident why rpr sensitized the nfo and wild type strains (Tables 1 and 4). As pCY5 did not sensitize either strain it is obvious that excess 3-methyladenine DNA glycosylase activity is not, in

itself, deleterious. It is possible, however, that the rpr effect is similar to that seen with E. coli alkA gene clones (Kaasen et al., 1986). Though not directly demonstrated, it has been suggested that alkA sensitization may be due to the depletion of rare tRNA molecules required for alkA translation, thereby diminishing the pool available for other DNA repair genes (Kaasen et al., 1986). We have not, as yet, sequenced rpr, thus precluding such an explanation for the rpr effect. Finally, since the xth nfo mutant was not viable on the MMS concentration used (Table 1) it could not determined whether rpr or tag sensitized this mutant. Logically, it would be expected that the sensitization would be even more pronounced in the E. coli xth nfo mutant.

It is clear that rpr does have certain limitations with respect to tag alkA and recA complementation. The tag alkA recA strain (MV2153) is highly sensitive to MMS and rpr did not restore MMS resistance even at a low level (Table 2). This experiment suggests that for rpr to be effective in complementing either a tag alkA or recA mutation the proteins encoded by the alternative genes must be functional.

The experiment designed to determine the effect of plasmid copy number on recA complementation served the following purpose: since rpr encodes a function which

sensitizes E. coli wild type strains to MMS we thought that a reduction in such activity might result in more efficient complementation by rpr. Table 3 shows that this was not the case. This is in direct contrast to data shown in Table 4 for rpr sensitization of PF1018 (E. coli wild type). That is, a reduction in the level of Rpr by cloning rpr on pACYC184 (i.e., pSM11) completely abolishes the MMS sensitivity. These data suggest that rpr has some characteristic that is harmful to wild type strains of E. coli, but if present in low copy, rpr sensitization is not observed. Whether this characteristic is due directly to Rpr activity per se, or some secondary action, is not known.

We have effectively eliminated one possible explanation for rpr complementation of an E. coli recA mutation. Data presented here indicate that rpr does not encode AP endonuclease activity. It can be concluded that rpr must complement the recA mutation by acting in some other capacity. Future work must concentrate on determining what that capacity is. The MMS sensitization of various strains by rpr presents interesting questions. Purification of Rpr and DNA sequuence analysis will aid in delineating the biochemical functions of Rpr.

EXPERIMENTAL PROCEDURES

Chemical reagents and enzymes. Methyl methanesulfonate (MMS) was purchased from Aldrich Chemical Company. Restriction endonucleases and T4 DNA ligase were purchased from Bethesda Research Laboratories.

Bacterial strains. All bacterial strains and the source of each are listed in Table 5.

Genetic manipulations and recombinant DNA techniques. Plasmid isolation, cloning procedures and all relevant genetic manipulations were performed as previously described (Maniatis *et al.*, 1982).

Media. Luria broth and minimal salts medium less glucose were as described (Maniatis *et al.*, 1982). Antibiotics were used at the following concentrations: ampicillin 50ug/ml, chloramphenicol 10ug/ml, kanamycin 50ug/ml, tetracycline 10ug/ml.

MMS sensitivity. Assay for MMS sensitivity was as previously described (Evensen and Seeberg, 1982). The concentration of MMS employed in the individual experiments varied depending on the strain being tested. Each experimental table contains specific information on the amount of MMS in the medium.

Plasmids. Plasmids used as cloning vectors were pUC18, pACYC184 and pBR322. Plasmids pCM1 and pJC859 contain the *E. coli recA* gene in pACYC184 and pBR322, respectively. Plasmids pCY5 (Sakumi *et al.*, 1986) (*tag*$^+$

in pUC8) and pYN1000 (Nakabeppu *et al.*, 1984a) (*alkA*$^+$ in pBR322) were kindly provided by Dr. M. Sekiguchi. Plasmids pSM4 and pSM2 harbor the *S. marcescens rpr* gene and have already been described (Murphy and Braymer, 1989). Plasmid pSM11 contains the 2.8kb *Bam* HI-*Bgl* II fragment from pSM2 in pACYC184.

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Table 1. MMS sensitization of *E. coli* strains deficient in AP endonuclease activity by *S. marcescens rpr* gene.

Strain/Plasmid		Efficiency of Plating[a] (%)
[b]PF11	pUC18	98
	pSM4	14.2
	pCY5	97.2
[c]PF111	pUC18	94.35
	pSM4	0.65
	pCY5	0.68
[d]PF1316	pUC18	87.4
	pSM4	0.58
	pCY5	89.5
[e]PF1317	pUC18	<0.5
	pSM4	<0.5
	pCY5	<0.5

[a]E.O.P. determined on Luria agar containing 0.035% MMS and appropriate antibiotic (kanamycin and/or ampicillin) at a concentration of 50ug/ml.

[b]*E. coli* wild type

[c]*E. coli xth* mutant

[d]*E. coli nfo* mutant

[e]*E. coli xth nfo* mutant

Table 2. Complementation of *E. coli tag alkA recA* mutation by individual DNA repair genes.

Strain/Plasmid		Efficiency of Plating[a] (%)
[b]MV2153	pUC18	<0.13
	pACYC184	<0.13
	pCM1 (*recA*$^+$)	<0.16
	pSM4 (*rpr*$^+$)	<0.16
	pCY5 (*tag*$^+$)	<0.15
	pYN1000 (*alkA*$^+$)	<0.2

[a]E.O.P. was determined on Luria agar containing 0.008% MMS, tetracycline (10ug/ml) and either ampicillin (50ug/ml) or chloramphenicol (10ug/ml).

[b]MV2153 is an *E. coli tag alkA recA* mutant.

Table 3. Effect of rpr gene dosage on complementation of *E. coli recA* mutation.

Strain/Plasmid		Efficiency of Plating[a] (%)
[b]χ2813	pUC18	<0.2
	pACYC184	<0.25
	pCM1	93.9
	pJC859	94.4
	pSM2	26
	pSM4	31
	pSM11	32

[a]E.O.P. was determined on Luria agar containing 0.008% MMS and either ampicillin (50ug/ml) or chloramphenicol (10ug/ml).

[b]χ2813 is an *E. coli recA* mutant.

Table 4. Sensitization of an *E. coli* wild type strain by *rpr* cloned on various vectors.

Strain/Plasmid		Efficiency of Plating[a] (%)
[b]PF1018	pUC18	82.24
	pBR322	82.17
	pACYC184	82.03
	pCY5	82.35
	pYN1000	71.29
	pSM2	59.83
	pSM4	7.5
	pSM11	81.50

[a]E.O.P. was determined on Luria agar containing 0.025% MMS and either ampicillin (50ug/ml) or chloramphenicol (10ug/ml).

[b]PF1018 is an *E. coli* strain which is wild type with respect to *tag alkA recA*.

TABLE 5. Bacterial strains and plasmids.

Strain	Relevant genotype	Source
S. marcescens		
ATCC 25419	wild type	Laboratory stock
E. coli K-12		
PF11	wild type	P. Foster
PF111	as PF11 but *xth*	P. Foster
PF1316	*nfo-1*::km^r	P. Foster
PF1317	*nfo-1*::km^r *xth*	P. Foster
PF1018	wild type	P. Foster
MV2153	*tag alkA recA*	M. Volkert
ℛ2813	*recA56*	NEB[a]
pUC18	amp^r	Laboratory stock
pBR322	amp^r tet^r	Laboratory stock
pACYC184	cam^r tet^r	Laboratory stock
pCM1	cam^r *recA*$^+$	This work
pJC859	amp^r *recA*$^+$	A.J. Clark
pCY5	amp^r *tag*$^+$	M. Sekiguchi
pYN1000	amp^r *alkA*$^+$	M. Sekiguchi
pSM2	amp^r *rpr*$^+$	Murphy and Braymer (1989)
pSM4	amp^r *rpr*$^+$	Murphy and Braymer (1989)
pSM11	cam^r *rpr*$^+$	This work

[a]New England Biolabs

CONCLUDING REMARKS

This dissertation details the molecular cloning and biochemical characterization of a DNA repair gene from Serratia marcescens. Genetic analyses have revealed that the gene, rpr, is unique in comparison to other prokaryotic DNA repair genes. The rpr gene was shown to complement two distinct E. coli DNA repair mutations for resistance to MMS. These mutations, recA and tag alkA, cannot be heterologously complemented by E. coli DNA repair genes (21). The protein encoded by rpr, a 42kD molecule, functions as a 3-methyladenine DNA glycosylase with an activity spectrum identical to that of E. coli TagI. This finding provided the biochemical explanation as to the mechanism of tag alkA complementation by rpr (21). Experiments showing that rpr complemented the recA mutation only for MMS resistance proved that rpr was not the S. marcescens analog of E. coli recA (20).

Additional studies indicated that Rpr is actively involved in DNA repair and does not act merely to prevent damage by MMS. That is, in the host cell reactivation (27) experiment rpr and tag were equally effective at reactivating bacteriophage lambda that had been exposed to MMS. These data notwithstanding, rpr is not a panacea for all E. coli mutants sensitive to MMS. For instance, rpr did not complement E. coli alkB or xth nfo mutations.

Furthermore, rpr was not capable of complementing a battery of E. coli ada mutations for resistance to MNNG. The inability of rpr to complement the xth nfo mutation strongly suggested that Rpr does not have an associated AP endonuclease activity. In fact, rpr actually sensitized E. coli xth and nfo mutants to MMS. This sensitization by rpr was also induced in E. coli wild type strains. Further proof that rpr is unique was provided upon Southern blot (32) analysis which showed that rpr does not share homology with cloned E. coli DNA repair genes.

While a reason for tag alkA complementation was found, the mechanism of recA complementation by rpr remains to be elucidated. Even so, some possible explanations have been eliminated. For example, it is clear that rpr does not induce the SOS response, since rpr does not complement the E. coli recA mutation for resistance to ultraviolet light. Secondly, it is unlikely that rpr is involved in recombinational repair as Rpr does not function as a DNA recombinase. What, then, are other plausible explanations for complementation of the E. coli recA mutation? It is possible that Rpr is replacing E. coli RecA in an unknown capacity. More specifically, if recA mutants are sensitive to MMS not only because of SOS repression, but because of the loss of RecA itself, perhaps Rpr can perform this secondary

RecA function. A second possibility is that Rpr acts to prevent DNA degradation by RecBCD (i.e., exonuclease V). This type of activity is characteristic of the lambda Gam protein. Perhaps the most interesting result from work involving the recA mutation is that rpr did not complement the triple E. coli tag alkA recA mutation. This implies that Rpr activity is not sufficient to restore MMS resistance in the absence of SOS response, Tag I and Tag II activity. Regardless, future work must be directed towards defining the mechanism of E. coli recA complementation by rpr. This should be initiated by testing the above three hypotheses.

It may be tempting to theorize that S. marcescens evolved the rpr gene in place of a recA gene, This, however, has not occured. In fact, the recA gene from S. marcescens has been cloned and sequenced and the encoded protein differs from E. coli RecA by only one amino acid. The S. marcescens recA gene fully complements the E. coli recA mutation (M. Benedik, personal communication). Thus, the rpr gene is not a substitute for recA in S. marcescens. Conversely, whereas recA from E. coli and S. marcescens are virtually identical, rpr does not share homology with recA from either organism. Of further interest is the fact that S. marcescens does not appear to possess DNA repair genes with homology to either E. coli tag or alkA. This would imply that the primary

function of Rpr is that of a 3-methyladenine DNA glycosylase.

S. marcescens, then, has two genes which can complement the E. coli recA mutation for MMS resistance. This phenomenon is not entirely unique. Haemophilus influenzae also has two genes, rec and fec, which also complement the pleiotropic effects of the E. coli recA mutation (1). These latter two genes, however, share DNA homology (1), while rpr and recA do not. It appears, though, that certain bacterial genera have evolved duplicate, albeit different, genes to repair identical types of DNA damage.

The observed sensitization of E. coli wild type, xth and nfo mutants is intriguing. It is most likely that sensitization of the E. coli xth mutant is due to excess 3-methyladenine DNA glycosylase activity, since tag and rpr have the same effect on the xth mutant. Sensitization of the E. coli wild type and nfo strains cannot be presently explained, although rpr gene dosage does play a major role in determining the degree of MMS sensitization.

This discovery of a new type of DNA repair system will add considerably to our understanding of DNA repair mechanisms in prokaryotes. Heretofore, such knowledge has relied almost solely on studies utilizing E. coli. Future

studies in this laboratory will include DNA sequence analysis which will augment investigation of *rpr* function.

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VITA

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Keith E. Murphy

Major Field: Microbiology

Title of Dissertation: Molecular Genetic and Biochemical Analyses of a DNA Repair Gene from *Serratia marcescens*

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

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